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Research Article

Changes on mutant pot chrysanthemums (*Dendranthema* × *grandiflora* Tzelev.)

Gulden HASPOLAT

Aegean Agricultural Research Institute, Mail Box: 9, 35660, Menemen, Izmir, Turkey; gulden.haspolat@tarimorman.gov.tr

Abstract

Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev) is one of the most important ornamental plants in the world that has the richest mutant varieties with numerous colors. The objective of this study is to determine the effective mutagen dose (EMD50) for creating variations by gamma irradiation. It is aimed to get a mutagenesis protocol that could develop new mutants in pot chrysanthemums. To determine the EMD50, rooted cuttings of brownish-red color 'Brandevil' variety were irradiated by gamma radiation at 0, 10, 20, 30, 40, 50 Gray (Gy) doses. According to the shoot lengths, EMD50 was calculated as 27 Gy. The mutation frequency was calculated as 4.8%. Some changes were observed for flower numbers per plant, plant heights and widths, shapes and colors of both flowers and leaves. The color changes varied from brownish-red to yellow and orange. Two different colors appeared in the same pot at some genotypes as well as form changes of flowers. The similarity of the mutants was determined by the hierarchical cluster dendrogram involving five groups. Various colors were obtained for leaves and flowers. Remarkable mutations of the selected mutants were multiplied by tissue culture.

Keywords: breeding; gamma irradiation; *in vitro*; mutation frequency; variation

Introduction

Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev), is a well known ornamental plant among the different world cultures and has different meanings with cultural significances. It has a large use such as cut flowers, pot flowers, and garden plants throughout the world (Anderson, 2006). Chrysanthemum was first cultivated as a flowering herb back in the 15th century BC in China (Shahrajabian *et al*., 2019). Interspecific hybrids under successive selections and management resulted from cultigen complex on chrysanthemums over 3000 years (Zhao, 2011). In Turkey, Chrysanthemum is mostly used as a cut flower and takes the fourth place most widely cultivated cut flower after dianthus, gerbera and rose (Kazaz *et al*., 2013; Kazaz *et al*., 2020).

It is extremely important for the chrysanthemum breeders to meet the demand of developing new varieties, for flower color and type, flowering time, stress tolerance, and post-harvest evaluations. Flower color and type variation are quite high in chrysanthemums. The presence of complex and variously shaped ray and disc flowers results in type variations. Crossbreeding and mutation breeding are traditional methods for obtaining variability alongside molecular techniques such as transgenic technology, genome editing and marker assisted selection (Schum, 2003; Datta and Janakiram, 2015; Su *et al*., 2019; Song *et al*., 2020). Mutation

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breeding on vegetatively propagated crops is applied mostly to ornamental plants and chrysanthemum has the richest mutant varieties (Nagatomi *et al*., 2000; Ahloowalia and Maluszynski, 2001; Van Harten, 2002; Zalewska *et al*., 2010; Melsen *et al*., 2021).

Chrysanthemum, is a self-incompatible cross-pollinated plant and has severe heterozygosity, complex genetic background, difficulty matching parents, selecting superior hybrid progenies causing complexity to the inheritance of genetic factors, coupled with frequent polyploidy (Ronald and Ascher, 1975; Zhao *et al*., 2006; Zhu *et al*., 2014; Yang *et al*., 2015; Zhang *et al*., 2018; Kumari *et al*., 2019). Because of these obstacles compared to cross breeding, mutation breeding has advantages inducing variability in vegetatively propagated crops. It also offers an advantage for improving of major traits within a short period of time (Nagatomi, 2001; Kunter *et al*., 2012; Shu *et al*., 2012; Singh and Bala, 2015).

Mutation breeding induced variants are remarkable to improve available genetic resources in modern agriculture. Spontaneous mutations have played an important role in the evolution of many garden chrysanthemums. In addition, another type of mutation is physical or chemical mutagens that cause further genetic improvement. The introduction of induced mutation is considerable in chrysanthemums since any mutation in dominant genes is easily expressed in the first generation. Thus the selection of mutations of directly perceptible characters like flower color, shape or size is generally directly can be put in commercial use (Zhenhua and Shouhe, 1995; Singh and Bala, 2015).

The objective of present study was to create variation in potted chrysanthemum with mutation breeding methods, find the effective mutagen dose (EMD50) in rooted cuttings, then observe morphological changes due to radiation treatment and create genetic variability among the population.

Materials and Methods

Irradiation treatments were carried out on rooted cuttings of the brownish-red colored pot type 'Brandevil' chrysanthemum variety. The study was conducted in the greenhouses at Bademler Village Agricultural Development Cooperative in Seferihisar, Izmir, Turkey.

Determining effective mutagen dose

For determining the effective mutagen dose (EMD50), 5 cm long rooted cuttings were irradiated at 0, 10, 20, 30, 40 and 50 Gray doses of gamma rays (Cobalt irradiator - ^{60}Co, Izotop, Ob-Servo Sanguis Co-60 Research Irradiator, Budapest, Hungary) in Turkish Energy, Nuclear and Mining Research Agency, Nuclear Energy Research Institute. The irridated cuttings were planted in pots (14 cm) and all the standard cultural operations were followed. Shoot length and shoot weight were determined on the 60th day of irradiated plants.

Irradiation at effective mutagen dose

After determination of EMD50, 2000 rooted cuttings were irradiated with gamma rays at the dose of EMD50. Plants were observed in M_1V_1 (2019) and M_1V_2 (2020) periods. The observations carried out on: Plant survival and loss rates (the ratio of died mutants to planted mutants), late flowering rate (ratio of flowering plants after one week later according to the population) color and type differentiations like spoon ray florets (Figure 5), compound flowers (two flowers on a flower stalk), non-flowering buds (blind bud), chimeric flowers and leaves, small (flower width: 2.5 cm ≥) and big flowers (flower width: ≥ 3.5 cm), dwarf plants (plant length: 15 cm ≥ and plant width: 18 cm ≥), doubled ray florets (number of ray florets raws: ≥ 10).

The mutation frequency (MF) of plants was considered in 2020 (MF: ratio of mutant plants to all irradiated plants). Mutant plants consisted of some changes on plants, leaf and flower abnormalities; color and form variations of flowers. Plant height (cm; from the soil surface to the uppermost part of terminal meristem) and width (cm; the widest aboveground plant growth width), number of shoots and lateral branches per plant,

the node number of the stem of the terminal shoot and lateral branches, internodal length (cm) of the stem of the terminal shoot and lateral branches were recorded (Anderson and Ascher, 2004). Number of leaves, leaf length (cm; the longest part of leaves) leaf width (cm; the widest part of leaves) and petiole length (cm; longest part of the petiole), leaf color, number of flowers per plant, flower width (cm; the widest part of the flower), flower length (cm; measurement of vertical part of flower), flower stem length (cm; the length between flower and leaf), number of ray floret type, number of ray floret raws and number of ray florets were collected in 2020 during anthesis. Somatic mutations of the flower color and chlorophyll variegations were mentioned as chimeras. Flower and leaf color of control and mutant plants were determined by using the "Methuen Handbook of Colour Chart" (Kornerup *et al*., 1978). The new color variations were isolated and propagated by tissue culture techniques.

Cultivation on tissue culture

The nodal explants (1 cm long with axillary bud) were used to isolate mutant shoots. First the explants were washed thoroughly in running tap water for 30 minutes and disinfected with 70% ethanol for 7 minutes and followed by surface sterilization with hydrogen peroxide (H_2O_2) solution (25%) for 7 minutes then washed thoroughly in sterile distilled water three times. Then the explants were planted on basal MS (Murashige-Skoog, 1962) medium supplemented with 3% (w/v) sucrose and solidified with 7.5 g L^{-1} of agar which included plant growth regulators 2 mg L^{-1} 6-Benzylaminopurine (BAP).

Statistical analysis

Analyzing EMD50, a completely randomized design (CRD) was employed and calculated EMD50 by linear regression statistical analysis using the data according to shoot lengths. Each treatment consists of four replications with 25 treated cuttings each. Changing ratios of genotypes according to the control group were obtained (*1*).

$$\%\ Change = \frac{Genotype - Control}{Control} x100 \quad (1)$$

Data were analyzed using the SAS-JMP software, version 7.0 (SAS Institute Inc., Cary, North Carolina, USA). According to the ward method, the dendrograms were created using cluster analyses to assess the similarity between mutant genotypes. According to the hierarchical cluster, the clustered data was analyzed through the one-way analysis of variance. The statistically significant groups in terms of the examined feature were grouped by Least Significant Difference (LSD) post hoc.

Results

During the calculation of effective mutagen dose (EMD50), while the gamma-ray dose increased, decreases were observed in shoot length and the weight of rooted plants (Table 1, Figure 1, Figure 2 and Figure 3). EMD50 was determined as 26.98 Gy as a result of linear regression analysis performed according to shoot lengths (Figure 1).

Table 1. Measured shoot length and plant weight values the 60th day after irradiation

Doses (Gy)	Shoot length (cm)	Plant weight (g)
Control	13.8	22.8
10	11.9	16.8
20	6.8	9.9
30	4.5	9.5
40	4.1	5.6
50	3.0	2.6

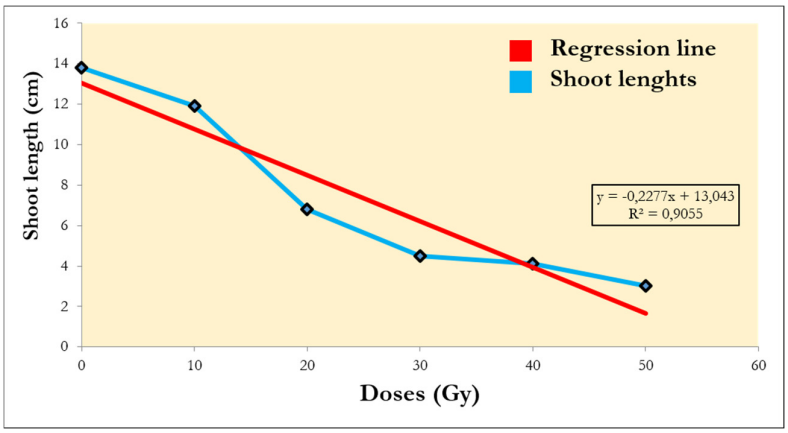

Figure 1. Variation of shoot length according to the doses

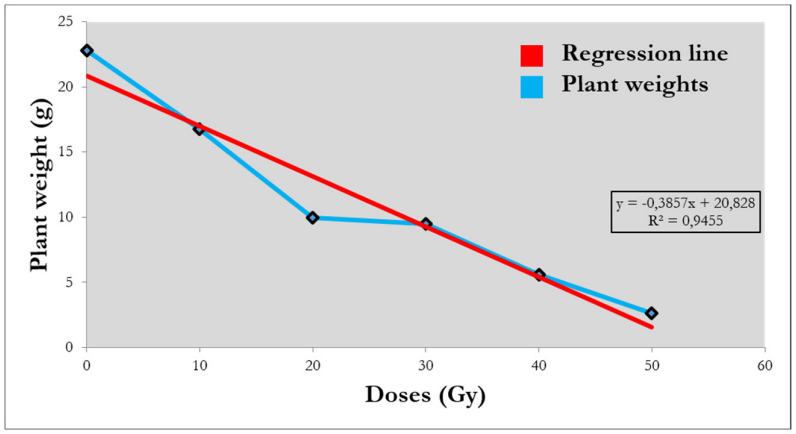

Figure 2. Variation of plant weight according to the doses

Figure 3. Variation of plant length according to the doses; (A). Control; (B). 10 Gy (Gray); (C). 20 Gy; (D). 30 Gy; (E). 40 Gy and (F). 50 Gy (bar=10 cm) (Photo: Burak Kunter)

After the determination of EMD50, 2000 rooted cuttings were irradiated at the dose of EMD50 as 27 Gy. Flowers were observed in the M_1V_1 and M_1V_2 periods. Color and form changes occurred in both years. Most of the variation determined as spoon ray florets (Figure 5) in the M_1V_1 period, while the loss rate was the highest in the M_1V_2 period, which can be interpreted as a consequence of irradiation effects. The survival percentage was 99.6 in the first year, then decreased to 71.5 in the second year. On the other hand, compound and chimeric flowers were inducted in the M_1V_1, while the chimera formations were observed only in leaves at

the M_1V_2 period. The rate of color changes of flowers was higher in the M_1V_2 (5.8%) period than in the M_1V_1 period (0.4%). Spoon ray floret formation was decreased in the M_1V_2 period, besides any compound flowers obtained in this period. The big and small florets rate had the same value in the M_1V_2 period (Figure 4). Even though the loss rate was higher in M_1V_2, the variations were more important regarding novel flower colors and shapes in one branch or whole plant than M_1V_1.

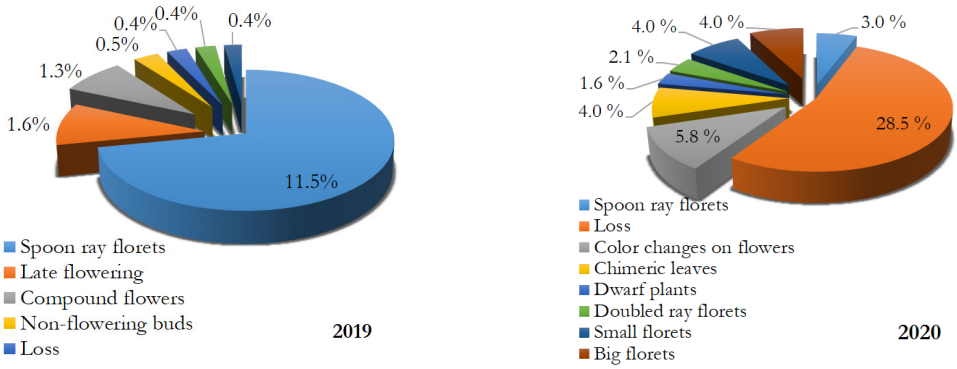

Figure 4. Changes of the plants in 2019 (M_1V_1 period) and 2020 (M_1V_2 period)

Figure 5. Spoon shaped ray florets (A) spoon florets with brownish red color; (B) orange colored spoon florets with flat ray florets in same pot; (C) spoon ray florets with brownish color (bar=1 cm) (Photo: Gulden Haspolat)

Some quantitative and qualitative characters were recorded and the mutation frequency was determined as 4.8% in the M_1V_2 period. The color of the control group's flowers was brownish-red at the young stage, then turned to lake red in matured flowers. Some changes in flower color were observed in the irradiated group up to light orange and vivid yellow, according to Kornerup *et al*. (1978).

The data clustered to obtain similarities between genotypes in terms of plant height and width, number of nodes per stem, internode lengths of the stem, number of lateral branches, the node number of lateral branches, internode length of lateral branches, shoot number, flower number per plant, flower width and length, flower stem length, number of ray floret type, number of ray floret raws, number of ray florets; leaf width and length, petiole length, leaf numbers per plant, flower and leaf colors. According to the cluster analysis, the population was divided into five groups, including 95 genotypes and mean values of control individuals. The number of genotypes in the clusters was: 30 genotypes in the 1st group; 18 genotypes in the 2nd group; 10 genotypes were observed in the 3rd group, 23 genotypes in the 4th group with control individuals; and 15 genotypes were located in the 5th group (Figure 6).

The dendrogram showed that G1 and G9 are the furthest genotypes while G4 and G11 are the nearest ones (Table 2). G1 took part in the first group, had a good plant shape with garnet brown flowers, more ray florets (130) and olive green leaves. G9 was located in the third group with the brownish red flower color and

had 89 ray florets with spoon and flat ray floret shapes. The genotypes G4 and G11 were in the 1st group with the similarities in flower numbers per plant, plant height and widths, same flower widths, shoot numbers and the number of ray floret raws.

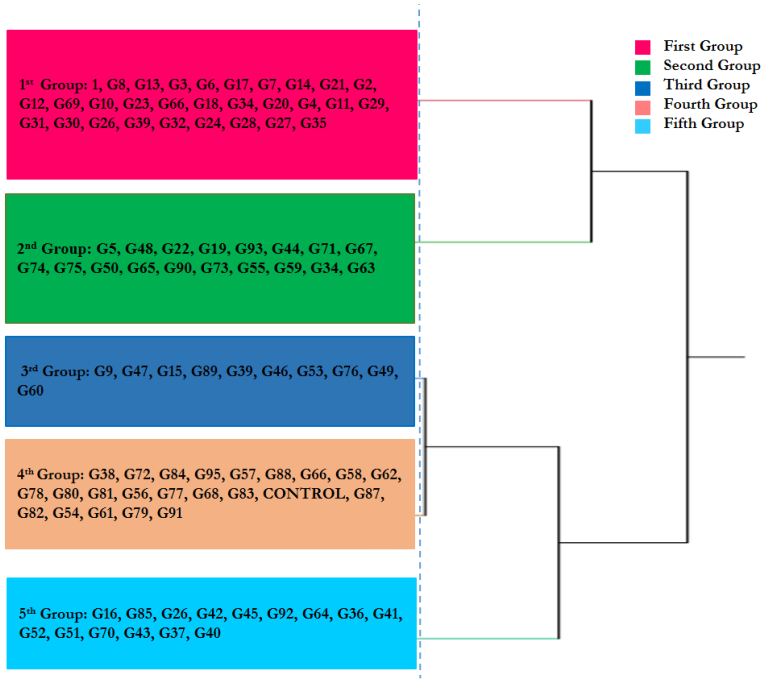

Figure 6. Dendrogram of mutant genotypes (by: the author)

Table 2. Distance of the genotypes according to the cluster groups

Genotype 1	Genotype 2	Distance
G4	G11	1,80
G3	G6	1,99
G57	G88	2,08
G10	G23	2,14
G84	G95	2,12
G16	G37	7,67
G9	G38	7,96
G9	G16	11,48
G1	G5	12,33
G1	G9	14,87

On the other hand, the mutant population had different flower shapes with different colors and leaves in the same pot for some genotypes. For example, genotype 14 had both orange and cardinal red flowers in addition the shoots with cardinal red flowers had chimeric leaves with greyish green color. Another example was genotype 28, with the vivid yellow and garnet brown florets in same pot. The genotypes 17 and 27 had orange and brownish red flowers in the same plants too (Figure 11).

In the mutant population, a decrease of 53.8% was achieved in plant height compared to the control, while 35.9% growth was achieved (Figure 7 and Figure 8). This variation was obtained in the leaf number with an increase (91.8%) and decrease (90.1%). While an increase of 51.1% was detected for the flower number 86.4% decrease was determined (Figure 7).

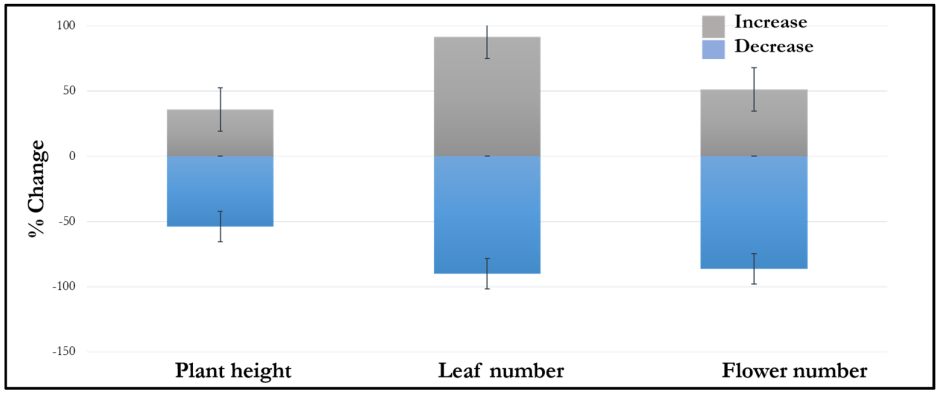

Figure 7. Changing ratios of the population according to the control for plant height, leaf number and flower number

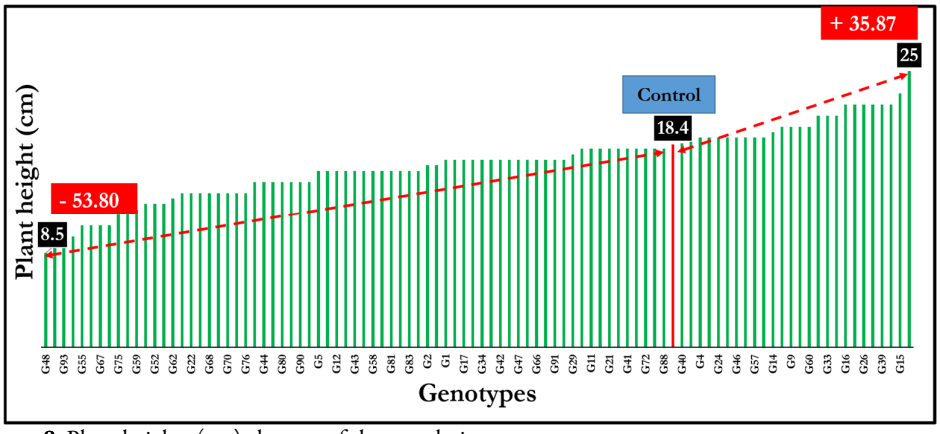

Figure 8. Plant heights (cm) changes of the population

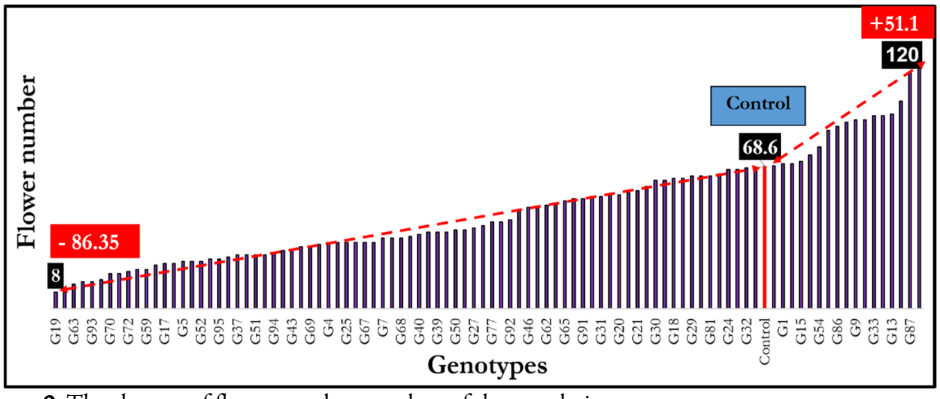

Figure 9. The changes of flower number per plant of the population

According to the one-way variance analysis in plant height, genotypes in the 1st and 3rd groups were prominent, while short-height genotypes were observed in the 2nd group. In addition, a remarkable increase in plant width was detected among the genotypes of the 3rd group. The plants in the 3rd group including 10 genotypes had the highest and widest plants among the whole population (Table 3; Figure 8).

While the shoot number of the genotypes in all groups got the highest value, the shoot number of the genotypes belonging to the fifth group took the last place. The mean shoot number was observed as 3.1 in the 4th group while 1.7 in the 5th group. The genotypes of the first group had the longest values according to the

internode lengths of the stem. For the number of lateral branches, the genotypes of the 3rd and 4th groups had the highest lateral branches (Table 3).

Table 3. Changes of the genotypes according to the cluster groups

Characteristics	Level	Least Sq Mean	Std Error	Characteristics	Level	Least Sq Mean	Std Error
Plant heigth (cm)	3A*	18.9	0.78	Plant width (cm)	3A*	32.9	1.58
	1AB	18.3	0.45		4B	26.7	1.04
	5BC	16.9	0.64		1B	25.0	0.91
	4C	16.6	0.51		2C	19.4	1.18
	2D	12.4	0.58		5C	18.6	1.29
Number of ray floret raws	4A*	8.1	0.41	Number of ray florets	5A*	138.5	8.52
	5A	7.9	0.51		3AB	123.8	10.44
	3ABC	6.6	0.63		1AB	120.8	6.03
	2B	6.4	0.47		4B	112.9	6.88
	1C	5.2	0.36		2B	110.9	8.52
Number of ray floret type	4A*	1.3	0.09	Number of flowers	3A*	71.4	6.36
	5AB	1.3	0.10		1B	56.6	3.67
	2AB	1.2	0.09		4B	53.8	4.19
	3AB	1.2	0.13		5C	30.1	5.19
	1B	1.0	0.07		2C	24.1	4.74
Flower width (cm)	3A*	3.8	0.17	Flower length (cm)	3A*	2.1	0.09
	5A	3.7	0.11		4B	1.8	0.06
	4B	3.3	0.11		5B	1.8	0.07
	1B	3.0	0.10		2C	1.6	0.07
	2C	2.6	0.12		1C	1.5	0.05
Number of lateral branches	3A*	12.6	1.15	Internode lenght of lateral branches (cm)	3A*	1.1	0.07
	4A	12.5	0.76		5B	0.7	0.06
	2B	7.6	0.85		4BC	0.6	0.04
	1BC	5.7	0.66		1C	0.5	0.04
	5C	5.0	0.93		2C	0.5	0.05
Shoot number	4A*	3.1	0.39	Internode lengths of stem	1A*	0.88	0.04
	1AB	2.3	0.34		2B	0.71	0.06
	2AB	2.1	0.44		4BC	0.63	0.05
	3AB	1.9	0.59		3BC	0.56	0.05
	5B	1.7	0.48		5BC	0.53	0.53

*Levels not connected by the same letter are significantly different, (LSD test, $p < 0.01$)

The genotypes of the 3rd group had the highest values of flower number per plant, flower length and internode length of lateral branches. The 3rd group was also had the widest flower values with the 5th group in terms of flower width. The mean flower width was 3.8 cm in the 3rd group, followed by the 5th group (Table 3). The M_1V_2 generation population decreased by -86.4% compared to the control plant group's flower number and a 51.1% improvement was achieved (Figure 7 and Figure 9).

All groups had more ray floret types, except for the first group. The first group had flat ray floret type, whereas the others had 2 or more ray floret types like flat, spoon or tubular shaped. The 1st, 3rd, and 5th groups had more ray florets per flower. The highest mean value of the ray floret number was 138.5 in the 5th group. Most groups had the highest number of ray floret raws except for the 1st and 2nd groups. The number of ray floret raws was changed between 3 and 13, while the control grup's mean ray floret raws was 7. The first group had the lowest value of (5.2) mean ray floret raws, while the 4th group's mean ray floret raws were 8.1 (Table 3).

The leaves and leaf numbers are as remarkable as the flowers in potted chrysanthemums. For this reason, we also discussed our plants in terms of leaf characteristics. The first and third groups had the highest number of leaves among the five groups. The highest mean leaf number was 190.4 in the 3rd group. The leaf numbers of genotypes were changed between 15 and 290. The control plants had 151 mean leaf numbers. When evaluated in terms of the changing rates due to control plants, the number of leaves was between -90.1% and 91.8% (Figure 7 and Figure 10). These variabilities in number of leaves result from the diversity created by the applied radiation doses in the plants. While for leaf and leaf petiole lengths the longest values were obtained in the 3rd, 4th and 5th groups, the genotypes of the 5th group had the widest leaves in leaf width. The shortest mean leaf and petiole length and leaf width were observed in the 2nd group (Table 4).

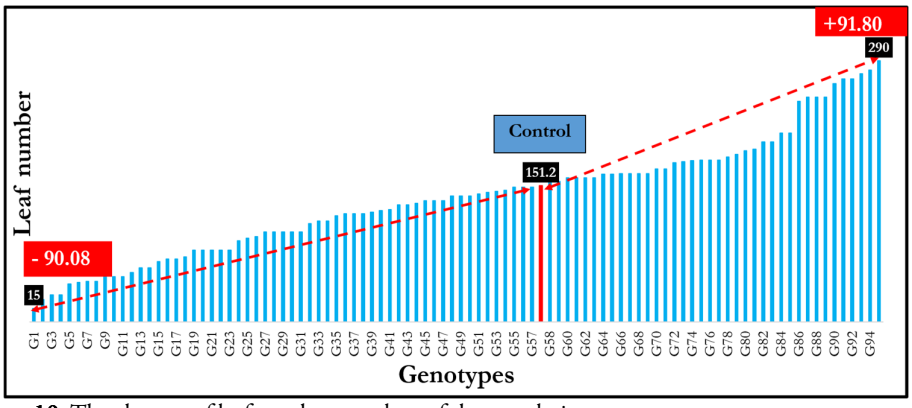

Figure. 10. The changes of leaf number per plant of the population

Table 4. Changes of the leaves of genotypes according to the cluster groups

Characteristics	Level	Least Sq Mean	Std Error	Characteristics	Level	Least Sq Mean	Std Error
Leaf width (cm)	5A*	2.8	0.10	Leaf length (cm)	5A*	5.5	0.33
	3B	2.3	0.13		3A	5.2	0.41
	1B	2.3	0.07		4A	5.1	0.27
	4B	2.3	0.08		1B	4.1	0.23
	2C	1.9	0.09		2C	3.2	0.30
Leaf number	3A*	190.4	17.45	Petiole length (cm)	5A*	1.1	0.09
	1AB	160.6	10.07		4AB	0.9	0.07
	4BC	140.5	11.51		3AB	0.9	0.10
	2C	121.2	13.01		1BC	0.8	0.06
	5D	71.2	14.25		2C	0.7	0.08

*Levels not connected by the same letter are significantly different(LSD test, $p < 0.01$)

Discussion

The results of the present study are most consistent with what other investigators have reported. The effective mutagen dose (EMD50) differs according to the plants, cultivars and the irradiated material, therefore it is of great importance to determine the most appropriate EMD50, for the cultivars in each mutation study. The EMD50 of the 'Brandevil' variety was determined as 26.98 Gy (Figure 1). The decreases were observed in shoot length and rooted plant weight while increasing gamma-ray dose. Similarly, the EMD50 of the 'Bindiya' variety was determined as 30 Gy dose according to Singh and Bala (2015) while Setia *et al.* (2020) found that 10 or 15 Gy doses were effective for inducing novel flower color variants on 'Purnima' variety. On the other hand, from Patil *et al.* (2015, 2017) EMD50 of 'Local Golden' variety was calculated between 2.5 and 3.0 Krad. They've also observed decreases in shoot length with increasing doses.

Kumari *et al*. (2013) indicated that flower head size and fresh weight decreased as the dose increased. The leaf abnormalities were observed in changes in leaf shape, leaf size, leaf margin, and leaf apex. Various flower color and shape changes were recorded in the form of chimeras. The yellow mutant was observed at 10 Gy and the quilled petals mutant occurred at 15 Gy. The color and shape changes on flowers and leaves were also observed in the present study. We got the leaf and flower variations in both shape and size. The chimeric leaves also had light color changes at leaf margins as well as leaf apex (Figure 11). The shape changes differed from small and big florets to spoon shaped ray florets. Also, as in the studies of Singh and Bala (2015) and Kumari et al. (2013), spoon ray floret formations (Figure 5) and flower size variations (Figure 11) were revealed.

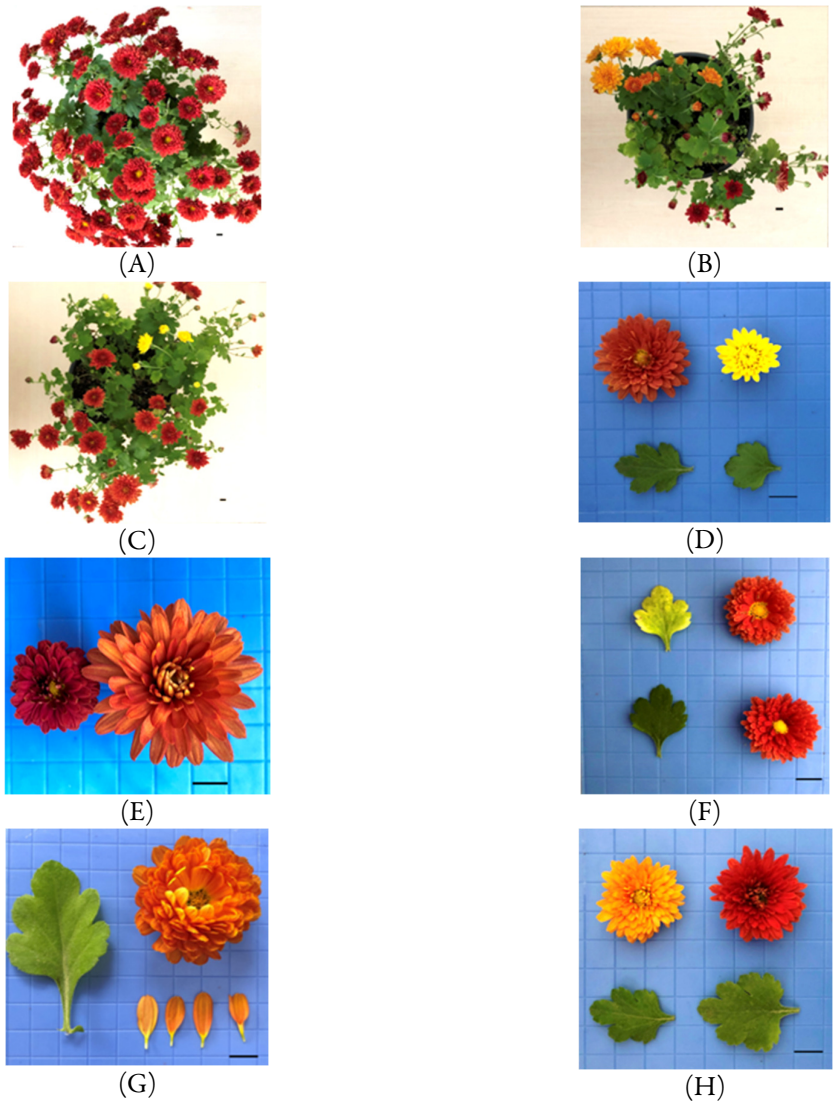

Figure 11. (A) Control plant; (B) The orange and brownish red flowers of Genotype 17; (C)-(D) The vivid yellow and garnet brown florets of Genotype 28; (E) Two types and color of flowers of Genotype 14; (F) The chimeric leaves of Genotype 24; (G) The golden yellow color flowers of Genotype 43; (H) The orange and brownish red flowers of Genotype 27 (bars= 1 cm) (Photos: Gulden Haspolat).

Singh and Bala (2015) recorded variations of the variety of 'Bindiya' and reported morphological abnormalities such as fused leaves with lower levels of chlorophyll after being exposed to the 30 Gy dose of gamma rays. While the original flower color is red, flower color mutants were of the nearest shades of red color

at 10 and 20 Gy doses. The ray florets were normal in control, whereas the ray florets were spoon-shaped, tubular and irregular in induced variants. In the present research, flower shape, colour and size variations were observed on irradiated plants, including spoon ray florets (Figure 5) although, the ratio of formation spoon shaped ray floret mutants was decreased in the second year.

The differences in the height and width of the plants are caused by the applied radiation. On the other hand, short plant height is an important feature in potted plants in terms of controlling plant height and obtaining compact plants. Patil *et al*. (2015, 2017) have considered that the plant height and number of flowers per plant of the 'Local Golden' variety was maximum in control compared to irradiated ones. There was no significant difference in the colour of florets of treated and control plants. In contrast, we had some genotypes with higher plants and more flowers than the control group with the changing ratios of 35.9% and 51.1% respectively (Figure 8 and Figure 9). We considered mutations in flower color and chlorophyll variegations. Moreover, we obtained flower colors from vivid yellow to shades of orange like Mandal *et al.* (2000). They detected chimeras on flower color and chlorophyll variegation on leaves in treated different doses of gamma rays on rooted cuttings of the 'Maghi' variety. They isolated and propagated these mutants successfully by using tissue culture techniques. We already propagated some of the mutants such as genotype 43 using tissue culture and obtained 3600 plants after subculturing 150 clones for 7 months of this genotype. On the other hand, the bud explants of vivid yellow-colored florets of Genotype 28 were isolated from the shoots and *in vitro* propagated and 50 plantlets are subculturing.

According to Lee *et al*. (2010), the morphological characteristics of leaves, the leaf length and the width of the 'Beakma' variety were decreased and petiole length was increased as the increment of dose. Similar changes like decreasing leaf number (Figure 10), length and width; formation of chimeric leaves, shape abnormalities of leaves were observed in our research at the 'Brandevil' variety. We had 18 genotypes among the mutant population, with the shortest leaf length and leaf petiole lengths. In addition, we observed small shaped apple green- and yellow-coloured leaves or yellow colours at leaf margins (Figure 11).

Setia *et al*. (2020) was observed seven colour mutants at 'Thiching Queen' and two flower colour variants in 'Purnima' cultivars. The leaf abnormalities were appeared in mutant populations exhibiting variation in flower colour, shape and size of leaves. Certain floral abnormalities were also observed in the inflorescence with an increase in irradiation dosage. Banerji and Datta (2003) indicated gamma-ray induced different types of flower head shape mutants. In our study, variable flower colours, shapes, sizes of leaves and plants were obtained similarly. We had different shaped and coloured flowers as well as chimeric leaves (Figure 11).

In the present study, we wanted to create diversity by applying gamma rays to the potted chrysanthemum variety and obtained mutants that could be used as new varieties with colour changes in flowers and leaves. Due to the lack of potted chrysanthemum variety in Turkey, such studies should be focused on in the near future. In addition, *in vitro* cultures are used to integrate into the breeding cycle to determine genetic diversity in a short time (Van Harten, 2002). Since *in vitro* techniques allow for rapid clonal propagation, desired results are obtained in selecting mutants (Datta, 2014). Important characteristics of mutants on one shoot or stem should be propagated by tissue culture techniques. Tissue culture techniques enabled the preservation and propagation of the mutants from bud explants.

Conclusions

The treatment of gamma-rays can be used to produce exclusive mutations from the chimeric tissues with new varieties to meet the demand of the floriculture market in *Dendranthema* x *grandiflora* Tzelev. Potential application of gamma-ray (^{60}Co) induced mutagenesis protocol developing noticeable and desirable mutants in chrysanthemum can be an effective way of breeding to establish new varieties. Most of the studies on

mutagenesis in chrysanthemums using gamma-rays with flower colour/shape and chlorophyll variegation in leaves. In this respect, induced mutagenesis is a very effective breeding method in chrysanthemum. Studies for each variety are important in determining the effective mutagen dose in mutation breeding. If the determination of the effective mutagen dose is missed, the same dose that provides a variation in one variety can be fatal for the other variety. In order to obtain suitable genotypes for our breeding target, EMD dose was determined to expand the variation in the 'Brandevil' variety. This variation in the criteria evaluated shows the suitability of the calculated EMD value and the genetic structure of the studied variety to the mutation. According to the results of researches, irradiation has variated plant height occurred changes both on flower parts and leaves. The useful changes can provide improving new varieties. From another point of view, tissue culture techniques are essential to isolate major mutations on a branch or flower and can be used successfully to develop new variants. Our suggestions to future researchers are combining tissue culture techniques with mutation breeding to obtain new novel characters homogeneously. To summarize of our results, mutation breeding is a modest way of discovering new variants with directly recognizable traits such as flower color, shape and size.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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